Attorney Docket No. TMT-10902/04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael T. Trese et al.

Serial No.: 10/068,314 Group Art Unit: 3763

Filed: February 6, 2002 Examiner: Matthew F. DeSanto

For: METHOD FOR VITREOUS LIQUEFACTION

SECOND SUPPLEMENTAL DECLARATION OF MICHAEL K. HARTZER, Ph.D.

I, Michael K. Hartzer, hereby declare as follows:

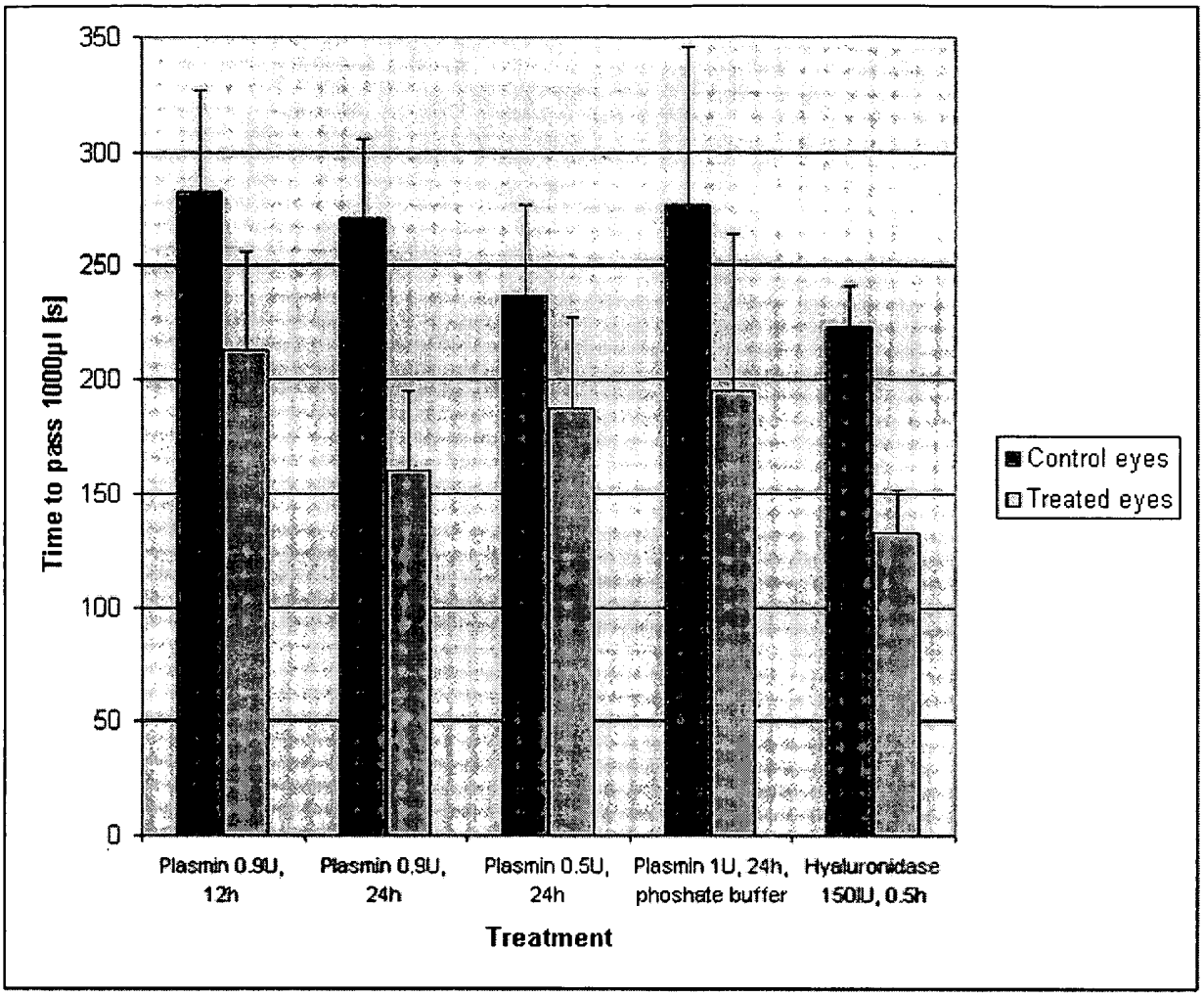
1. I reaffirm my statements made in my previous declarations of May 9, 2003 and October 17, 2003 with the correction that the May 9, 2003 declaration incorrectly identifies the complex as streptokinase-plasmin and my declaration of October 17, 2003 corrects this discrepancy to correctly identify it as a streptokinase-plasminogen complex.

2. In order to assess the efficacy of plasmin injections to induce vitreous liquefaction, I undertook a study to assess enzymatic vitreous liquefaction by plasmin through a measurement of the speed of vitreous removal from rabbit eyes using a standard small gauge system. My collaborators on this study were M.A. Hermel of the Department of Ophthalmology, University of Aachen; Aachen, Germany; J. Prenner of Associated Retinal Consultants, Royal Oak, Michigan; Wendy Dailey of NuVue Technologies, Keene, New Hampshire; and my co-inventor M.T. Trese.

3. We performed these experiments by isolating plasminogen from human plasma by affinity chromatography and activating the plasminogen to form active plasmin using streptokinase in a molar ratio of 1:10 relative to plasminogen. Resulting plasmin activity was

assessed spectrophotometrically. SPF New Zealand white rabbits (2 kilogram) were anesthetized followed by intravitreal injection of 0.1 milliliter of solution containing one of: 0.9 units of plasmin in balanced saline solution, 0.5 units of plasmin in balanced saline solution, 1 unit of plasmin in phosphate buffer, or 150 units of hyaluronidase. The fellow eye of each rabbit received an injection of 0.1 ml balanced saline solution. Hyaluronidase is known to reduce vitreous liquefaction but not a posterior vitreous detachment and therefore served as a positive control. Thirty minutes after the intravitreal injection of 150 units hyaluronidase, and either 12 hours or 24 hours later for the plasmin experimental group, a vitrectomy was performed under anesthesia by the following procedure. A single 25 gauge pars-plana sclerotomy was created and a 25 gauge cutter (Storz) was used to remove the vitreous using a standardized protocol of 200 millimeters suction, 1 cut per second. To avoid infusion artifacts during the procedure, the eye was allowed to collapse and the low cutting rate served merely to minimize incarceration of the tip. The time required to remove the vitreous was measured using calibrated suction tubing, and average flow rates were calculated. The rabbits were sacrificed after surgery and statistical evaluation was performed using the Wilcoxon paired signed rank test.

4. The results of this study indicated that the time required to remove 1 milliliter of vitreous was significantly reduced by either plasmin or hyaluronidase compared to balanced saline solution injected into fellow eyes. I believe the data clearly indicates plasmin is effective not only at the concentrations of plasmin indicated but also the concentrations of less than 0.4 units to liquefy vitreous and ongoing experimentation supports my belief. While vitreous liquefaction occurs more slowly than the creation of a posterior vitreous detachment, I believe this methodology will prove clinically beneficial particularly when used with small gauge cutting systems. The results of this study are summarized in the following graph.



5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Michael K. Hartzer, Ph.D.

Date: 5/7/04

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